A clasped embrace

Dinshaw J. Patel

HUMAN chorionic gonadotropin (hCG) is a placental glycoprotein hormone that induces the secretion of progesterone to sustain the early weeks of pregnancy[1]. But despite its central role in reproductive physiology, we have had little definitive information about the global folding of hCG, the interactions between its α- and β-subunits, the elements of its structure that interact with its receptor, and the molecular events that lead to signal transduction.

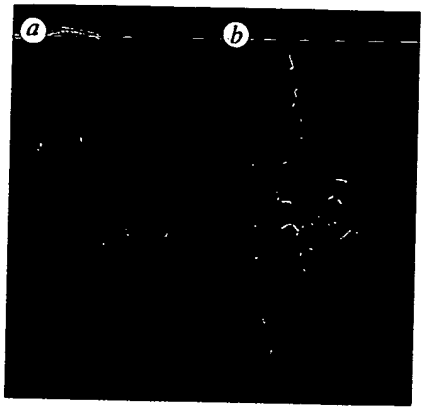

FIG. 1*a*, Ribbon diagram of the hCG heterodimer with α-chain (5–89) in red and β-chain (2–111) in blue. The amino and carboxy termini of the chains are labelled and the disulphide bridges are omitted. *b*, The Cα backbone trace of the hCG heterodimer viewed in a similar orientation to *a*. The α-chain is in red, the β-chain in blue; residues β(90–111) are in light blue and the disulphide bridges are in yellow. (All figures courtesy of Hao Wu).

This barren landscape is now immeasurably brightened by the appearance of two reports of the X-ray crystal structure of hCG, one on page 455 of this issue[2], the other in *Structure*[3]. They go a long way towards linking structure and biological function for the family of glycoprotein hormones that stimulate ovarian and testicular function. The folding topology of hCG is most unusual in that it has unexpected folding motifs, interlocking subunit–subunit interactions and symmetry relationships, as well as a surprisingly high ratio of protein surface to hydrophobic core. Not least, the newly solved structure will open up fresh avenues for structure-based approaches to the design of drugs for both contraception and the stimulation of fertility.

Human chorionic gonadotropin is a noncovalent heterodimer composed of a 92-amino-acid α-subunit (with five disulphide bridges and two *N*-linked glycosylation sites) and a 145-amino-acid β-subunit (with six disulphide bridges and two *N*-linked and four *O*-linked glycosylation sites)[1]. Carbohydrates account for 30–35 per cent of the total mass of hCG, and crystals[4,5] have been successfully grown following hydrogen fluoride treatment that results in a roughly 50 per cent reduction of the carbohydrate content of the hormone[6]. Lapthorn *et al.*[2] have solved the hCG structure at 3.0 Å resolution, using multiple isomorphous replacement combined with maximum entropy solvent flattening techniques[2]. A less conventional approach was taken by Wu *et al.*[3], who produced the selenomethionyl protein in mammalian cells, crystallized it under anaerobic conditions and applied multi-wavelength anomalous diffraction measurements to generate the hCG structure at 2.6 Å resolution. This higher resolution structure also allowed the identification of 64 solvent molecules. The good news is that the same global fold has emerged from these two independent investigations; both groups have been able to trace residues 5–89 in the α-subunit and residues 2–111 in the β-subunit, and have defined the intermolecular interactions that stabilize the hCG $\alpha\beta$ heterodimer.

The heterodimer adopts an elongated shape consisting primarily of β-sheets with the two subunits intertwined into each other (Fig. 1). The core structure of each subunit contains a cystine knot motif[7,8], where a disulphide bridge connecting two strands penetrates into an eight-residue circle generated by a pair of disulphide bridges connecting two other strands. Extending out from the cystine knot in the α- and β-subunits are a pair of highly twisted hairpin loops, in almost parallel planes, which project in one direction, and a long, flat hairpin loop which projects in the opposite direction. The unexpected similarity of the global folds of the α- and β-subunits is best visualized by superpositioning the subunits as shown in Fig. 2*a*. The subunit superposition is striking in the cystine knot region (Fig. 2*b*), whereas differences are observed in the size, tip dimensions and pairing alignments within related hairpin loops. The long loop in the β-chain (blue, Fig. 2*a*) is much more open than its counterpart in the α-chain (red, Fig. 2*a*), with the latter having a sharp turn in the β-sheet at a proline-rich segment.

The reciprocal alignment of α- and β-subunits at the dimer interface generates a quasi-dyad symmetry axis (Fig. 1). The subunits are intimately associated over their interacting surfaces, and in the process bury a surface area of some 4,000 Å^2. The intermolecular interactions are of the β-sheet type at the heart of the interface, as well as in a segment where the disulphide-tethered carboxy-terminal tail

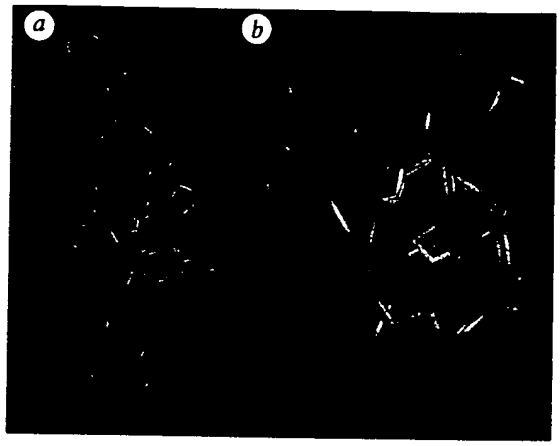

FIG. 2 Superposition of individual subunits. *a*, View of the α-subunit (in red, with magenta disulphide bridges) and β-subunit (blue with yellow disulphide bridges) of hCG following superposition of Cα positions in the central cystine knot of both subunits. *b*, Enlargement of *a* showing the common folds that are adopted by the cystine knot segments of the α- and β-subunits.

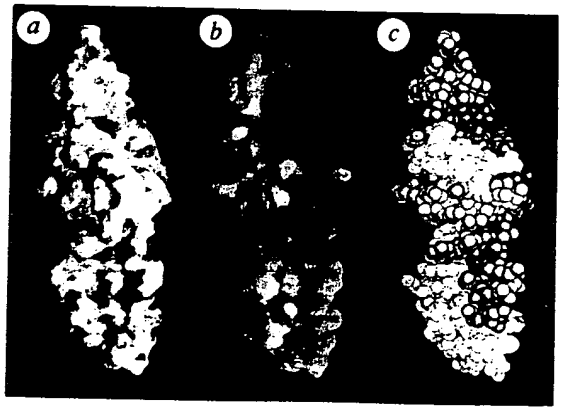

FIG. 3 The receptor-binding face of the hCG heterodimer. *a*, View showing the surface curvature with the convex, concave and planar surfaces in green, grey and white respectively. *b*, View showing the surface electrostatic potential, the most positive regions being blue and the most negative regions red. *c*, Space-filling view of the heterodimer (α-chain in pink, β-chain in blue), emphasizing the residues involved in receptor binding (α(88–89) in magenta, β(94–111) in green) and in signal transduction (Asn 52 and its attached carbohydrate in yellow).

of the β-subunit (light blue in Fig. 1*b*) embraces a long loop of the α-subunit. This 'seat-belt' arrangement has implications for possible *in vivo* folding pathways for hCG αβ heterodimer formation, because this process will be governed by the precise order of disulphide bridge formation. Another striking feature of the hCG heterodimer structure is the large surface-to-volume ratio, which results in a one-layer structure with a minimal hydrophobic core at the dimer interface.

The cystine knot motif and the loops emanating from it, as well as the seat-belt arrangement which clasps segments of the α- and β-subunits, should turn out to be characteristics of other members of the glycoprotein family of hormones. These include luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone, all of which are secreted by the pituitary. These hormones have a common α-subunit and homologous but distinct β-subunits, hCG being most closely related to luteinizing hormone. The hCG structure should provide insights into potential αβ heterodimer alignments in all three relatives.

Both hCG and luteinizing hormone have the same G-protein-coupled receptor, with distinct sites on the hormones being implicated in binding and signalling functions[9]. The receptor has a long extracellular domain extending out from a seven-helix membrane-spanning segment; the coupled G-protein is involved in activation of adenylyl cyclase to produce progesterone. A view of the surface curvature for the receptor-binding face of hCG is shown in Fig. 3*a*. The electrostatic surface potential of this face is markedly positive (blue in Fig. 3*b*), and should complement the negative potential associated with the acidic residues on the extracellular domain of the receptor. Further, residues α(88–89) (magenta, Fig. 3*c*) and β(94–111) (green, Fig. 3*c*) that have been implicated in receptor binding[10–12] are adjacent to one another on this face of hCG.

Equally interesting is the adjacent location on the same face of the *N*-linked Asn 52 glycosylation site which may be involved in signal transduction[13]. This Asn 52–$(GlnNAc)_2$ site, which is located in a depression on the hCG surface, is in yellow in Fig. 3*c*.

The determination of the hCG structure is a landmark among studies of the architecture and interactions of glycoprotein hormones involved in regulating reproductive physiology. The intertwined nature of the αβ heterodimer interface and its minimal hydrophobic core, coupled with the unprecedented seat-belt wrapping of a tethered segment of one subunit around a segment of its partner in the heterodimer, opens up new challenges for those investigating protein folding. The central involvement of hCG in the early stages of pregnancy makes it a key molecule whose function can be modulated through selective binding of agonists to stimulate fertilization or of antagonists to induce contraception. Structure-based drug design strategies can now be initiated to address these goals.

Finally, the stage is set for attempts to crystallize and solve the structure of the complex of hCG and its extracellular binding domain on the receptor. That research agenda could eventually provide insights into hormone–receptor interactions on the pathway to signal transduction. □

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SOLVATION DYNAMICS

Wet chemistry

James T. Hynes

WHEN a chemical reaction occurs in solution, the transition state barrier must be crossed *en route* from reactants to products. Chemists have long known that the height of this barrier, which exponentially affects the reaction rate, can be extraordinarily dependent on the solvent, particularly for reactions in a polar solvent in which there is charge displacement and thus strong electrostatic interaction between the reactants and the solvent molecules[1]. But solvation dynamics should also be important. Water, the commonest of solvents, is a case in point: how fast can its molecules reshuffle to suit the changing charge of the reaction intermediates? On page 471 of this issue, Jimenez *et al.*[2] show experimentally that the first, inertial response — tilting or twisting of the solvent molecules, rather than diffusive, reorientational motions — dominates aqueous solvation dynamics.

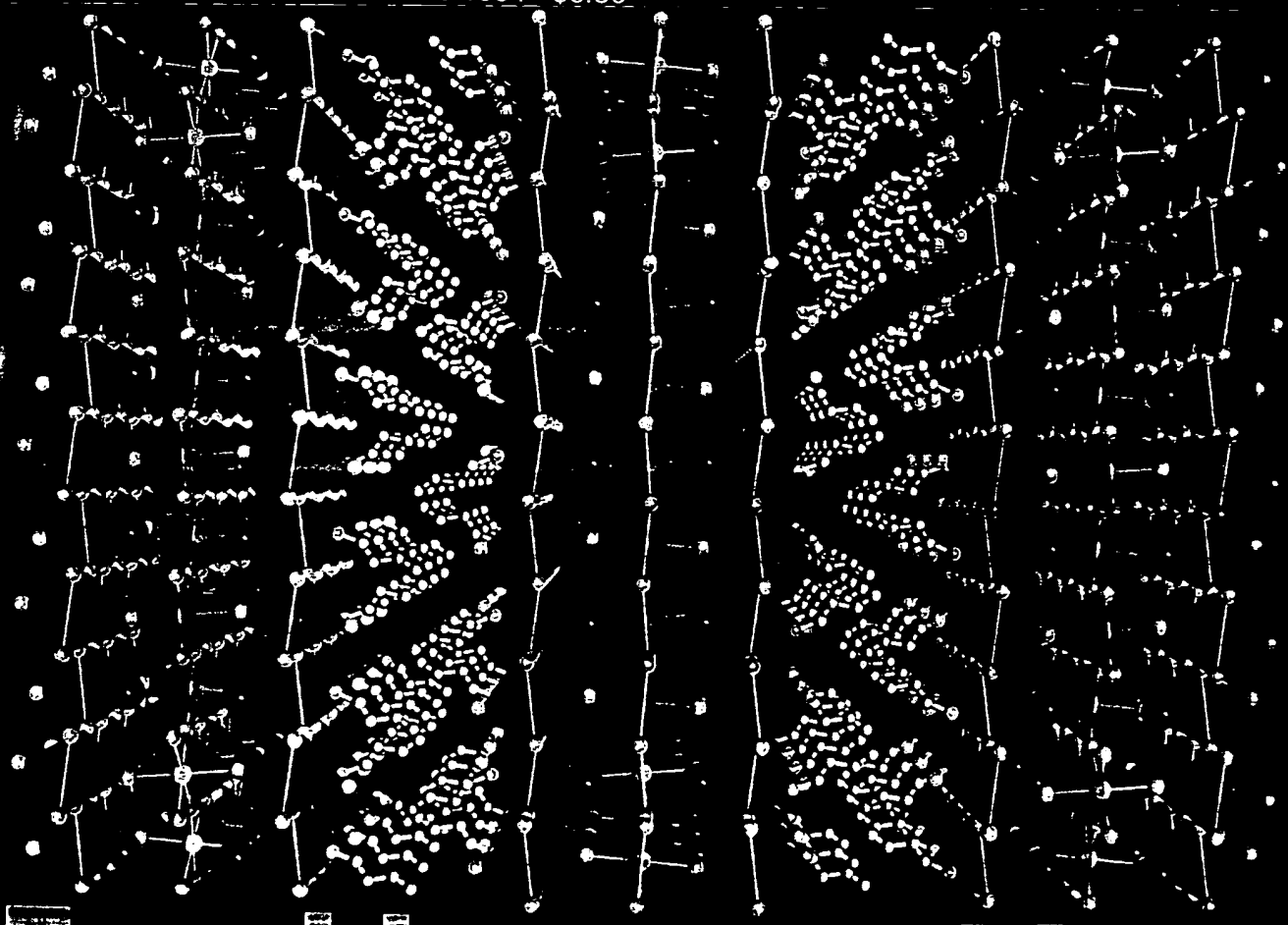
The exploration of solvation dynamics is of fairly recent vintage. For the past decade and a half, experimental and theoretical chemists have been trying to go beyond the standard notion of 'equilibrium solvation', instead probing just what the molecules are doing as the reaction progresses through the transition state and how this influences the rate.

To put this in context, consider how the well-known transition state theory[1] views a solution reaction, illustrated here by the bimolecular substitution reaction $Cl^- + CH_3Cl \rightarrow ClCH_3 + Cl^-$ in water[3,4]. In effect, it is supposed that as the new bond is formed and the old one is broken, and especially as the electrical charge is internally redistributed, the surrounding solvent molecules are always equilibrated to the reaction solute system $[ClCH_3Cl]^-$ as transit through the transition state occurs — this is the equilibrium solvation assumption mentioned above. Equivalently stated, it is assumed that the water solvent dynamics are fast compared with the (very brief) time spent by $[ClCH_3Cl]^-$ in the critical barrier region. If this condition is violated (and it is[4]), then the rate constant depends not only on the barrier height but also on the non-equilibrium dynamics of the solvent. And the actual rate constant will be less than transition state theory would predict. This can be understood in terms of the 'friction' that the reaction system experiences because of the non-equilibrium state of the solvent in the barrier passage; the larger that friction, the greater is the departure from the conventional theoretical rate[5,6].

Just how fast can a solvent equilibrate to a changing charge distribution of a solute within it? A powerful experimental avenue to answer this question is the study of time-dependence fluorescence (TDF) by ultrafast laser spectroscopy[7,8]. Typically, a dye molecule in solution is initially electronically promoted to an excited state whose charge distribution is different from that of the ground state. But now the solvent molecules are out of equilibrium with the new charge distribution, and must begin their dynamic readjustment to reach equilibrium. When the solute's excited state is fluorescent, this process can be followed by the time-dependent shift of the fluorescence frequency, the dynamic Stokes shift. In a real sense, this probe is the dynamic — and hence more complex — generalization of the various static spectroscopic probes which have long been exploited to characterize solvent polarity directly[1].

Early theoretical models of TDF were based on simple, nonmolecular, dielectric continuum descriptions of the solvent,

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Volume 369 No. 6480 9 June 1994 $8.50



Tunable perovskites